

# Dietary studies in birds: testing a non-invasive method using digital photography in seabirds

**Davide Gaglio<sup>1\*</sup>, Timothée R. Cook<sup>1,2</sup>, Maëlle Connan<sup>3</sup>, Peter G. Ryan<sup>1</sup> and Richard B. Sherley<sup>4,5,\*</sup>**

*<sup>1</sup>Percy FitzPatrick Institute, DST-NRF Centre of Excellence, University of Cape Town, Rondebosch 7701, South Africa; <sup>2</sup>Institute of Ecology and Environmental Sciences, Evolutionary Eco-physiology Team, University Pierre et Marie Curie, Bâtiment A–7<sup>ème</sup> étage, 7 quai, St Bernard, 75005 Paris, France; <sup>3</sup>DST-NRF Centre of Excellence at the Percy FitzPatrick Institute, Department of Zoology, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa; <sup>4</sup>Environment and Sustainability Institute, University of Exeter, Penryn Campus, Penryn, Cornwall, TR10 9FE, United Kingdom; <sup>5</sup>Animal Demography Unit, Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa.*

\*Corresponding authors, email: (DG) [swift.terns@gmail.com](mailto:swift.terns@gmail.com); (RBS) [r.sherley@exeter.ac.uk](mailto:r.sherley@exeter.ac.uk)

## Summary

1. Dietary studies give vital insights into foraging behaviour, with implications for understanding changing environmental conditions and the anthropogenic impacts on natural resources. Traditional diet sampling methods may be invasive or subject to biases, so developing non-invasive and unbiased methods applicable to a diversity of species is essential.

2. We used digital photography to investigate the diet fed to chicks of a prey-carrying seabird, and compared our approach (photo-sampling) to a traditional method (regurgitations) for the greater crested tern *Thalasseus bergii*.

3. Over three breeding seasons, we identified >24,000 prey items of at least 47 different species, more than doubling the known diversity of prey taken by this population of terns. We present a method to estimate the length of the main prey species (anchovy *Engraulis encrasicolus*) from photographs, with an accuracy < 1 mm and precision ~0.5 mm. Compared to regurgitations at two colonies, photo-sampling produced similar estimates of prey composition and size, at a faster species accumulation rate. The prey compositions collected by two researchers photo-sampling concurrently were also similar.

4. Photo-sampling offers a non-invasive tool to accurately and efficiently investigate the diet composition and prey size of prey-carrying birds. It reduces biases associated with observer-based studies and is simple to use. This methodology provides a novel tool to aid conservation and management decision-making in light of the growing need to assess environmental and anthropogenic change in natural ecosystems.

**Key-words:** diet, digital photography, non-invasive monitoring, prey-carrying birds, rarefaction curves, *Thalasseus bergii*, regurgitation

## Introduction

Dietary studies are essential to understand animal ecology, temporal changes in the environment, and to establish sustainable management strategies for natural resources (Jordan 2005). In complex natural systems, top-predators can act as indicators of environmental conditions, and their diet, in particular, can provide important information on prey species abundance, occurrence and size, which may reflect processes over short time-frames (e.g. Suryan *et al.* 2002; Parsons *et al.* 2008). As such, outcomes from diet studies are important tools for monitoring changes in demographic parameters or behaviour, themselves a product of changing diet (Sherley *et al.* 2013). Moreover, dietary studies can provide powerful indicators of anthropogenic impacts and environmental change on food-webs (e.g. Piatt *et al.* 2007; Green *et al.* 2015), facilitating conservation biology and ecosystem-based management (Grémillet *et al.* 2008; Sherley *et al.* 2013). The importance of monitoring diet thus demands the development of simple, efficient, non-invasive methods applicable to a diversity of species.

Numerous techniques exist to investigate bird diets (Jordan 2005; Inger & Bearhop 2008; Karnovsky, Hobson & Iverson 2012). Invasive techniques include induced regurgitations (Diamond 1984), stomach flushing of live birds (Wilson 1984), application of neck-collars on chicks (Moreby & Stoate 2000) and the dissection of birds collected specifically for this purpose (Doucette, Wissel & Somers 2011). These methods describe short-term diet composition accurately (González-Solís *et al.* 1997), despite some errors introduced by differential prey regurgitation or digestion (e.g. Jackson & Ryan 1986). More recent biochemical methods involving isotopic, lipid and DNA analyses provide complementary approaches, but generally cannot be used alone due to their coarse taxonomic resolution (Karnovsky, Hobson & Iverson 2012). Moreover, these approaches typically require disturbance or capture of birds, which can impact their physiology and behaviour (e.g. Ellenberg *et al.* 2006; Carey 2009).

Accurate, non-invasive diet sampling is therefore required to give fine-scale indicators of prey availability or prey selection. One of the least non-invasive methods is to observe birds carrying visible prey with binoculars or video recording systems, from a safe distance. This typically involves birds feeding offspring or incubating partners (e.g. Safina *et al.* 1990; Redpath *et al.* 2001; Tornberg & Reif 2007). Such studies are generally limited to assessing chick diet, but have the potential to reveal changes in prey communities (Anderson *et al.* 2014). However, observer-based diet studies are subject to several

methodological limitations (Cezilly & Wallace 1988; González-Solís *et al.* 1997; Lee & Hockey 2001) calling for further development of this approach.

Digital photography represents an excellent alternative tool to study the diet fed to chicks of prey-carrying birds, because 1) there is virtually no limit to the number of pictures that can be taken, 2) species identification is possible in most cases, 3) prey can potentially be measured accurately and precisely, 4) images can be re-analysed without loss of data quality, i.e. samples do not deteriorate over time and 5) storage is simple. Over the last decade, the use of digital photography for dietary studies has included the use of camera-traps to investigate the diet of nesting raptors (García-Salgado *et al.* 2015; Robinson *et al.* 2015), and the combined use of digital compact cameras with spotting scopes (digiscoping) to assist prey identification (made primarily by observations) for Caspian terns (*Hydroprogne caspia*) and common murrelets (*Uria aalge*) (Larson & Craig 2006, Gladics *et al.* 2015). However, both techniques have limitations including poor image quality and difficulty in capturing images of birds carrying prey in flight or during fast delivery to chicks (see Larson & Craig 2006, García-Salgado *et al.* 2015).

Recent advances in performance and price reductions of digital single lens reflex (DSLR) cameras combined with autofocus telephoto lenses makes digital photography an affordable option for prey identification, even for birds in flight. In the last few years, DSLRs have been used opportunistically to identify items carried by a variety of birds (e.g. Woehler *et al.* 2013; Gaglio, Sherley & Cook 2015, Tella *et al.* 2015) but a systematic approach and an accurate method to estimate prey dimensions are lacking. We developed a standardised application of digital photography using DSLR cameras and telephoto lenses to investigate chick diet composition and prey size in prey-carrying birds. We tested the method on the colonial breeding greater crested tern *Thalasseus bergii* in South Africa. We compared the efficacy of photo-sampling to the more traditional used regurgitations (Walter *et al.* 1987) using prey identified to species level collected from chicks, and assessed the accuracy and precision of length measurements of the main prey made from photographs. We also evaluated the potential for observer bias in this system. Finally, we discuss the validity of applying our non-invasive approach to any prey-carrying bird and the potential to develop a simple and effective tool-box to accurately identify and estimate the size of any carried item.

## 96 **Methods**

### 97 **STUDY SPECIES AND SITES**

98 The greater crested tern (hereafter ‘tern’) is distributed from the Namibian coast eastwards to the central  
99 Pacific. It feeds mostly at sea by dipping onto the surface or plunge diving up to ca 1 m (Crawford,  
100 Hockey & Tree 2005). During breeding, adults usually return from foraging with a single prey item,  
101 which is either offered to the partner during courtship or delivered to the offspring (Crawford, Hockey &  
102 Tree 2005). In South Africa, the sub-species *Thalasseus bergii bergii* breeds mostly on islands in the  
103 Western Cape (Crawford 2003). Since 2008, Robben Island (33°48’S, 18°22’E), Table Bay, has hosted  
104 the largest southern African colony, reaching ~13,000 breeding pairs in 2010 (Makhado *et al.* 2013). A  
105 few hundred pairs breed in the Eastern Cape, mostly on Seal Island (33°50’S, 26°17’E), Algoa Bay  
106 (Makhado *et al.* 2013). We studied their diet at both Robben and Seal Islands.

107

### 108 **PHOTO-SAMPLING**

109 We investigated the diet of breeding terns at Robben Island during 2013 (February–June), 2014  
110 (January–June) and 2015 (February–June) and at Seal Island during June 2015. Adult terns returning  
111 with prey were photographed from a vantage point of 50–80 m from the edge of their colony (Fig. 1a).  
112 At Seal Island (~300 pairs) we were able to photograph all adults returning to the colony during our  
113 photo-sampling sessions. At Robben Island, colonies were much larger (> 6,000 pairs) so we could not  
114 photograph all individuals. However, every attempt was made to not bias selection to individuals  
115 carrying particularly conspicuous prey items. The distance to the flying birds ranged between 6.5 and 25  
116 m. Total sampling effort represented ~ 50 h of photography per year. For each individual, we typically  
117 took a sequence of 3 photos (a “photo set”) for identification and prey measurements (Fig. 1b). We found  
118 by trial and error that 3 images provided the best trade-off to balance processing time with obtaining at  
119 least one sharp image. To avoid biasing the results and maintain independence among photo sets, ad-hoc  
120 image analysis was performed for each sampling session to discard repeated photo sets of the same  
121 adults carrying the same prey item. Recurrent birds were identified using distinguishable feather patterns,

presence of colour or metal rings, type and position of prey in the bill while flying, and distinctive markings on the prey.

Photos were taken using Canon 7D and 7D Mark II cameras, fitted with Canon EF 100–400 mm f/4.5-5.6L IS USM zoom lenses. We set the cameras to (i) shutter speed priority (1/2500 s); (ii) automatic ISO (or aperture priority mode that provided shutter speeds of at least 1/2500 s); (iii) high-Speed Continuous Shooting; (iv) Autofocus on AI Servo (for moving subjects) using the AF point expansion; and (v) large Jpeg file format for high-speed recording. We set the telephoto lens to autofocus, the image stabilizer to on and the closest focal point to 6.5 m to increase autofocus speed.

## IDENTIFICATION OF PREY SPECIES

All blurred or otherwise non-identifiable images (due to e.g. distance, an unfavourable position of prey in the bill or lighting) were discarded. From the remaining photographs (e.g. Fig. 1), we determined the numerical abundance (Duffy & Jackson 1986) of prey (usually at species level) using fish guides (Smith & Heemstra 2003; Branch *et al.* 2010) and assistance from experienced observers (see Acknowledgements). In some instances, good quality photographs contained prey that could not be identified (< 0.01% of total prey items). For example, some adults returned with pieces of fish flesh, possibly originating from kleptoparasitism disputes or scavenging. These images were excluded from our analyses. Approximately 45% of photo sets were suitable for prey identification; there was no evidence of bias towards particular prey types among discarded images.

## ESTIMATION OF PREY STANDARD LENGTH

Dietary studies of piscivorous birds commonly measure the standard length (SL) of the fish (length from the tip of the snout to the posterior edge of the hypural plate) to compare prey size (Barret 2002, Smith & Heemstra 2003). We estimated SL from photographs for anchovy *Engraulis encrasicolus*, the most common species in the tern's diet. As prey tended to flex to differing degrees in the adults' bills, direct SL measurement from the image underestimates fish length. Thus, we estimated SL from measurements of individual body parts (eye diameter, operculum width and head width, all measured dorsoventrally),

which were less distorted in the image and generally in a plane parallel to the bird's bill and the camera (Figs 1b and 2).

To do this we first assessed the accuracy of predicted SLs based on these morphological measurements using cross-validation by fitting log-linear allometric regressions to a training dataset ( $n = 50$ ) and comparing model predictions to a test dataset ( $n = 20$ ) of anchovies measured by hand (see Appendix S1). Next, we measured 37 additional anchovies with Vernier callipers (to the nearest 0.1 mm) and then photographed them held in the bill of a dead tern, for which the culmen length was known (Fig. 2 in Appendix S1). For each image, we used the 'line selection tool' in ImageJ (Schneider *et al.* 2012) to estimate eye diameter ( $\hat{E}$ ), operculum width ( $\hat{O}$ ) and head width ( $\hat{H}$ ) for each fish by scaling the pixel length in the image to (1) the length of the dead tern's culmen (62.1 mm; measured with Vernier callipers), (2) the mean culmen length for this species (61.2 mm,  $n = 128$ ; Crawford, Hockey & Tree 2005) and (3) the minimum and maximum recorded culmen lengths (range: 54.5–67.6 mm, Crawford, Hockey & Tree 2005). We used the estimates of  $\hat{E}$ ,  $\hat{O}$  and  $\hat{H}$  to obtain three estimates of SL ( $\widehat{SL}$ ) using the log-linear allometric regressions (see also Appendix S1), and calculated their arithmetic mean (combined  $\widehat{SL}$ ) and used this value in further analyses (since it was generally most accurate; Appendix S1).

To determine the accuracy ( $\gamma$ ) of the combined  $\widehat{SL}$  estimates from the images, we compared them to the known SL of each fish. We defined the mean percentage accuracy ( $\bar{\gamma}$ ) of the combined  $\widehat{SL}$  estimates as:

$$\bar{\gamma} = \frac{100}{n} \sum_{i=1}^n \left( 1 - \frac{|SL_i - \text{combined } \widehat{SL}_i|}{SL_i} \right)$$

(eqn 1)

where  $i$  indexes each of the  $n = 37$  fish. As the absolute difference was computed, both overestimates and underestimates of e.g. 2% would yield  $\gamma = 98\%$ . In addition, we assessed the mean difference between the known SLs and the combined  $\widehat{SL}$  estimates using permutation tests with 10,000 Monte Carlo iterations (*perm* library v. 1.0-0.0 for R).

To determine the precision (or repeatability) of the method, we repeated the measurement process in ImageJ to obtain six  $\hat{E}$ ,  $\hat{O}$  and  $\hat{H}$  values and the corresponding combined  $\widehat{SL}$  values for 17 of the 37 fish

(using a known length on the ruler in each photograph). We calculated the combined  $\widehat{SL}$  as above and used this to assess precision. Precision ( $\tau$ ) was defined as:

$$\tau_{f,j} = \left| \left( \frac{1}{n} \sum_{j=1}^n combined \widehat{SL}_{f,j} \right) - combined \widehat{SL}_{f,j} \right|$$

(eqn 2)

where  $j$  indexes each of the  $n = 6$  combined  $\widehat{SL}$  values for the  $f = 17$  fish. We report mean precision (in mm) of all ( $6 \times 17 = 102$ ) values of  $\tau_{fj}$ .

In addition, we examined whether either precision or accuracy were influenced by the SL of a fish. For accuracy, we used a linear model of the form:

$$logit(\gamma_i) = \alpha + \beta \times SL_i + \varepsilon_i$$

(eqn 3)

where  $\alpha$  and  $\beta$  are estimated from the data,  $\gamma_i$  are the accuracy estimates (as proportions),  $SL_i$  the known standard length for fish  $i$  and  $\varepsilon_i \sim N(0, \sigma)$  the residual error, with  $\sigma$  estimated from the data. For precision we used a linear-mixed model (LMM: *lme4* library for R) of the form:

$$\tau_{fj} = \beta \times SL_{fj} + \delta_{fj} \times \eta_j + \varepsilon_{fj}$$

(eqn 4)

where  $\beta$  is the fixed effect parameter,  $\eta_j \sim N(0, \varsigma)$  the random effect parameter,  $\varepsilon_{fj} \sim N(0, \sigma)$  the residual error,  $\delta_{fj}$  the vector of fish IDs,  $\tau_{fj}$  the vector of precision values and  $SL_{fj}$  the vector of known standard lengths for each measurement  $j$  of fish  $f$ , with  $\beta$ ,  $\sigma$  and  $\varsigma$  estimated from the data.

Finally, we used the above approach to estimate SL of the prey in a subset of the digital images collected in the field where the bird's bill and the head of the prey were clearly visible and approximately parallel to the camera (Fig. 1b). For each image, we used combined  $\widehat{SL}$  and assumed the length of the bird's culmen to be 61.2 mm (see above).

## COMPARISON BETWEEN PHOTO-SAMPLING AND REGURGITATION-SAMPLING

To compare photo-sampling and regurgitation-sampling, we collected images of adults carrying prey and regurgitations from chicks concurrently on 18 and 19 April 2015 at Robben Island (photo-sampling



effort: 600 min) and on 9 June 2015 at Seal Island (photo-sampling effort: 132 min). Regurgitates were collected from the ground, while chicks were inside a pen during ringing operations (chicks often regurgitate when disturbed). Prey were later identified from whole-prey or diagnostic prey remains resistant to digestion such as otoliths and squid beaks using Clarke (1986), Smith & Heemstra (2003), Smale, Watson & Hecht (1995), Branch *et al.* (2010) and the Port Elizabeth Museum's reference collection. Prey items that were not identified mainly consisted of fish flesh and were excluded from our analysis. The SL of whole anchovies collected from regurgitations was measured using a ruler.

We compared the number of prey items from different taxa between methods using  $\chi^2$  tests and assessed differences in the estimated anchovy SLs using permutation tests (10,000 iterations) for each island separately as the SL variance between islands was heterogeneous (Levene's test:  $W_{(1,164)} = 5.8$ ,  $p = 0.017$ ).

We examined prey diversity using sample-based rarefaction curves as these allow for standardized comparison across collections that differ in sample size (Gotelli & Colwell 2001). Using 1,000 random permutations of both the photo-samples and regurgitations from 18 and 19 April 2015, we produced curves of the mean ( $\pm$  asymptotic 95% confidence intervals, CI) species accumulation rate (species identified per sample made). We then compared this rate at samples sizes of  $n = 190$ . In addition, by fitting a Generalised Additive Model (GAM) to the photo-sample means and by assuming equal accumulation rates for extrapolation, we also compared the predicted species accumulation rate for regurgitations to the mean rate for photo-sampling at  $n = 1500$ . The chosen sample sizes approximate those obtained in the field.

Finally, to evaluate any possible observer effect on photo-sampling, two different researchers (observer-A and observer-B) simultaneously collected photographs at Robben Island on 18 and 19 April 2015. The two observers used the same equipment (Canon 7D Mark II camera, Canon 100–400 mm lens) and had similar experience in wildlife photography. All other procedures were the same as described above. We compared the samples from the two observers using  $\chi^2$  tests. Unless otherwise stated, all means are presented  $\pm 1$  SD and all statistics were performed using R v.3.2.1.

## Results

### PHOTO-SAMPLING VS. REGURGITATION-SAMPLING

In total ~160,000 photos were taken during the three breeding seasons on Robben Island, yielding images of 24,211 prey items identifiable to species (96%, 48 species) or family (98%, 49 families) level (total of 51 prey taxa; Table 1). During the regurgitation comparison trial at Robben Island, we identified 27 species from 1,510 photo-samples compared to 11 species from 198 regurgitated prey items. At Seal Island, we identified 11 species from 157 photo-samples and 6 species from 103 regurgitated prey items (Appendix S2). The mean species accumulation rate at 190 samples was 0.075 (95% CI: 0.058–0.089) for photo-sampling and 0.057 (95% CI: 0.053–0.058) for regurgitations; however, at this sample size, the 95% CIs overlapped (Fig. 3). The number of species predicted from 1,500 regurgitations was 23.4 (based on the GAM extrapolation) versus 27.0 for photo-sampling (Fig. 3). The diet composition of main prey did not differ significantly between the two methods for Robben Island ( $\chi^2 = 47$ , d.f. = 42,  $p = 0.26$ ) or Seal Island ( $\chi^2 = 18$ , d.f. = 15,  $p = 0.26$ ; Table S3 in Appendix S2).

### ACCURACY AND PRECISION IN ESTIMATING ANCHOVY STANDARD LENGTH

Mean SL of the 50 anchovy used to calculate the allometric regressions between the morphometric measurements (training set) was  $109.6 \pm 13.5$  mm (range = 83.3–130.5 mm), similar to the 20 anchovy in the test set (SL  $112.8 \pm 3.0$  mm; range = 107.6–116.8 mm). The predicted  $\widehat{SL}$ s of the test set predominantly fell within the 95% prediction intervals for all three specific body part models (Fig. S1, Appendix S1). The mean accuracy ( $\bar{y}$ ) for the combined  $\widehat{SL}$  was  $97.9 \pm 1.7\%$  (range 93.0–99.9%) for the training set and  $97.3 \pm 1.8\%$  (range 92.5–100%) for the test set. Accuracy was not affected by SL in either case (linear models:  $p > 0.05$ , see Appendix S1).

The mean SL of the 37 photographed anchovy was  $113.4 \pm 6.7$  mm. With the culmen length of the dead tern (62.1 mm) as the reference, mean accuracy ( $\bar{y}$ ) for the combined  $\widehat{SL}$  was  $98.3 \pm 1.5\%$  (range 93.8–100%), yielding a mean combined  $\widehat{SL}$  of  $114.0 \pm 7.1$  mm (Table S2 in Appendix S1). With the species' mean culmen length (61.2 mm) as the reference, the mean combined  $\widehat{SL} = 112.7 \pm 7.0$  mm ( $\bar{y} = 98.1 \pm 1.5\%$ , range 92.2–99.9%; Fig. 4, Table S2). The length of a fish (actual SL) did not influence the accuracy in either case (linear models:  $p > 0.05$ , Fig. 4) and neither of the combined  $\widehat{SL}$ s differed

significantly from the actual SL (permutations tests:  $p > 0.05$ ). The mean accuracy ( $\bar{y}$ ) reduced to 88.9 ( $\pm 3.3$ )% and 91.3 ( $\pm 3.2$ )% for the minimum (54.5 mm) and maximum (67.6 mm) recorded culmen lengths respectively (Table S2) and these combined  $\widehat{SL}$  series did differ significantly from the actual SLs (permutations tests:  $p < 0.001$ ; see Appendix S1).

The mean precision of the combined  $\widehat{SL}$  estimates was 0.52 ( $\pm 0.38$ ) mm or 99.6 ( $\pm 0.3$ )%, with an absolute range of 0.02–1.58 mm or 98.6–99.99%. Precision was not related to the actual SL of the fish being measured (LMM:  $\chi^2 = 0.02$ ,  $p = 0.89$ ).

## COMPARISONS OF PREY SIZE BETWEEN PHOTO-SAMPLING AND REGURGITATIONS

At Robben Island, 116 anchovy from photo-samples (10% of anchovy photographed) and 20 from regurgitates (12%) could be measured, while at Seal Island, the corresponding values were 21 (18%) and nine (9%) respectively. Overall, the anchovy were longer at Seal Island (mean = 120.3  $\pm$  8.2 mm,  $n = 30$ ) than at Robben Island (91.2  $\pm$  13.2 mm,  $n = 136$ ;  $p < 0.001$ ; Fig. 5). For Robben Island, the mean combined  $\widehat{SL}$  of anchovy in the photo-samples was 91.3  $\pm$  13.6 mm compared to 90.8  $\pm$  11.1 mm for regurgitates (Fig. 5). At Seal Island, they were 121.6  $\pm$  9.3 mm and 117.4  $\pm$  3.6 respectively. The SL estimates from the two methods did not differ statistically for either Robben Island ( $p = 0.85$ ) or Seal Island ( $p = 0.21$ ).

## COMPARISON BETWEEN OBSERVERS

We identified 1,510 prey items of 22 species from the photographs taken by observer-A and 1,625 of 21 species from observer-B. Prey composition did not differ significantly between the two ( $\chi^2 = 72$ , d.f. = 64,  $p = 0.23$ ). However, three species were not recorded in common; observer-A photographed one horsefish Congiopodidae sp. and one eel Ophichthidae sp., while observer-B recorded three individuals of Cape hake *Merluccius capensis*.

## Discussion

Photo-sampling offers an effective, low-impact alternative to traditional diet studies for birds that carry prey items in their bill, with accurate prey identification and size estimates possible. Samples can be

acquired quickly and equivalent diet compositions obtained with relatively low effort (Fig. 3). In three breeding seasons, we sampled 24,211 prey items and identified 51 prey taxa (Table 1) with this approach; the most comprehensive diet analysis for terns in southern Africa prior to our study identified 25 species from 1,311 regurgitated prey items in 10 breeding seasons (1977–1986; Walter *et al.* 1987). Despite ~55% of photos being discarded, our approach yielded an order of magnitude more samples and identified twice as many species, with minimal disturbance to breeding birds.

The photo-sampling approach has several other advantages over traditional diet sampling. First, terns often regurgitate only the posterior body and caudal fin of a fish, making identification of similar species difficult (McLeay *et al.* 2009). Photo-sampling records the entire prey, and if there is doubt as to the identification, images can be shared easily with global experts or on specialized websites (e.g. I-spot). Second, photo-sampling can be used in a range of situations (e.g. on land or from a boat), by one individual (collection of regurgitations often involves many people), with minimal training in photography (cameras can be pre-set). Third, the photographic equipment is relatively affordable and once purchased can be used for several years, at multiple colonies and for several species. Also, although processing the photographs can be time-consuming, taking about 30 min for an average of 100 prey identified, the images can be stored and analysed multiple times if needed, without the loss of data quality or metadata (e.g. date and location).

Possible drawbacks associated with photo-sampling include the repeated photography of prey items, especially those with long handling times, leading the frequency of these items being over-estimated. This is predominately a problem in larger colonies, where it is difficult to follow the fate of individual prey items, and one that could be countered using delays (e.g. 5 mins) between photosets. When only a subset of prey is sampled, large or conspicuous prey items may induce an observer bias if they are easier to photograph, more readily identified to species level or more interesting to the photographer. Training photographers to randomise the photo-sampling as much as possible should help reduce this potential bias. Differences in photographic experience between different observers could create a potential bias and should be examined in future studies. Photo-sampling is difficult in bad weather (strong wind, rain or mist) and this may also introduce bias in some situations. Finally, one constraint of our study is that photo-sampling was applied to study chick diet. Although this can provide important insights into

changes in prey communities (Anderson *et al.* 2014), it may not always represent adult diet, or diet outside the breeding season (McLeay *et al.* 2009). We thus suggest implementing indirect methods such as measuring stable isotope ratios in e.g. blood and feathers of adults (Inger & Bearhop 2008) concurrently with photo-sampling. Moreover, applying both methods concurrently on marked individuals would allow the development of trophic discrimination factors in wild animals (Newsome *et al.* 2010).

More broadly, ecologists now use digital photography to study animals across a wide range of taxa (e.g. Morrison *et al.* 2011; Marshall & Pierce 2012; Gregory *et al.* 2014). Opportunistic observations have documented novel behaviours and trophic interactions (e.g. Gaglio, Sherley & Cook 2015; Tella *et al.* 2015), suggesting that standardised approaches to study species bringing items to a known location have great potential for ecological monitoring. This approach could also be applied to a diversity of taxa in addition to birds that carry prey (e.g. carnivores bringing prey to their offspring, or ants and termites carrying items to their nests). In any of these applications photo-sampling could provide high quality photographic data to complement the now extensive use of camera-traps.

The ecological information provided by prey size is almost as important as prey species, giving information on the targeted prey cohort and the predator's energetics. We demonstrated that prey size (anchovy SL) can be estimated accurately (~98%) and precisely (~99%) from images. The approach could be used with a wide variety of predators and prey species to eliminate biases associated with *in situ* visual observation (Lee & Hockey 2001). Even if photo-sampling is unlikely to obtain measurements as accurately or precisely as regurgitated/dropped prey, the sample size from photo-sampling is always likely to be greater than the number of prey found undigested. A crucial step to estimate absolute prey size is identifying a reference object (e.g. culmen, eye diameter) of known size, to provide a scale for prey measurements. These reference objects should be chosen carefully and the degree to which the selected trait varies within the population assessed to constrain and minimise errors where possible (see Results). Additional studies could photograph birds of known bill length, age and sex (e.g. colour banded individuals) with prey held with different angles to the body and compare larger numbers of observers photo-sampling concurrently to further quantify the errors associated with prey measurements. For prey species that are not distorted in images (e.g. some insects do not bend over a bird's bill), size can be

estimated directly and even when absolute estimates are not possible, the method still can be used to assess changes in relative prey size, allowing for spatial and temporal comparisons.

Crucially, the photo-sampling method caused little if any disturbance to the nesting birds. Distances from animals can be selected to balance each species' sensitivity against image quality. The opportunity to record the number and size of prey brought to offspring remotely and in real time without influencing behaviour, allows for accurate monitoring of temporal variability. For threatened or declining species (e.g. many seabirds; Croxall *et al.* 2012), such non-invasive methods can help elucidate functional links between population dynamics, environmental variability and anthropogenic pressures (Saraux *et al.* 2011). Incorporating these observations into detailed information on species composition and energy content for energetic models offers great potential for indicators of long-term and large-scale ecosystem change (Furness & Cooper 1982). Furthermore, with standardized protocols, digital images can be shared easily using digital platforms (e.g. I-spot, Google Images) to facilitate global collaborations (e.g. González-Solís *et al.* 2011; Lynch *et al.* 2015), encourage community involvement in citizen science projects (e.g. Newman *et al.* 2012), and develop data archives to answer as yet unforeseen questions. Given the growing need to assess environmental changes and human impacts on natural ecosystems (Hobday *et al.* 2015), our methodology offers a novel tool for collaborative efforts in conservation.

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## Data accessibility

All data used in this article are available in the Supplementary materials or Dryad Digital Repository (Gaglio *et al.* 2016) <http://dx.doi.org/10.5061/dryad.j647p>.

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503

## 504 **Supporting Information**

505 Additional Supporting Information may be found in the online version of this article.

506 **Appendix S1. Additional methods and results for estimation of prey standard length.**

507 **Appendix S2. Results of the comparison between photo-sampling and regurgitation.**

508

509 **Table 1.** Prey families in the greater crested tern diet identified by photo-sampling on Robben Island during the  
510 2013, 2014 and 2015 breeding seasons. N = number of prey items identified.

Prey type	Family	Species	N
<b>Fish</b>	Engraulidae	1	16206
	Dussumieriidae	1	2557
	Scomberesocidae	1	1658
	Syngnathidae	2	866
	Clupeidae	1	545
	Carangidae	2	409
	Gonorynchidae	1	351
	Atherinidae	1	198
	Mugilidae	1	117
	Merlucciidae	1	76
	Pomatomidae	1	67
	Soleidae	Unid.	63
	Champsodontidae	1	58
	Clinidae	Unid.	63
	Clinidae	5	25
	Holocentridae	1	47
	Nomeidae	2	46
	Triglidae	Unid.	43
	Blenniidae	Unid.	38
	Myctophidae	1	23
	Gobiidae	Unid.	9
	Gobiidae	1	23
	Scombridae	1	22
	Scyliorhinidae	Unid.	16
	Macrouridae	Unid.	12
	Congridae	1	12
	Coryphaenidae	1	10
	Sebastidae	Unid.	6
	Gobiesocidae	1	6
	Trichiuridae	2	9
	Tetraodontidae	Unid.	5
	Cheilodactylidae	1	4
	Ophichthidae	Unid.	5
	Bregmacerotidae	Unid.	4
	Ophidiidae	1	3
	Ophidiidae	Unid.	1
	Sparidae	2	3
	Congiopodidae	Unid.	2
	Berycidae	1	2
	Centriscidae	1	1
	Chlorophthalmidae	1	1
	Batrachoididae	1	1
	Aulostomidae	Unid.	1
<b>Cephalopods</b>	Loliginidae	2	54
	Sepiidae	1	85
	Octopodidae	1	11
<b>Crustaceans</b>	Squillidae	1	244
	Brachyura*	Unid.	2
	Portunidae	1	1
	Palinuridae	1	3
<b>Insects</b>	Gryllidae	1	191
	Gryllotalpidae	1	2
	Sphingidae	1	2
	Sphingidae	Unid.	1
	Coleoptera **	Unid.	1

511 \*Infraorder, \*\*Order

## Figure Legends

**Fig. 1 a)** Examples of capturing a photo-sample of an adult greater crested terns carrying prey to the colony without causing disturbance to nesting birds and **(b)** the resulting close-up image of the prey used for identification (anchovy) and standard length measurements. **From c to n:** Examples of tern prey items: **c)** sardine *Sardinops sagax*; **d)** Atlantic saury *Scomberesox saurus*; **e)** multi-prey load (3 anchovy and 1 sardine); **f)** dolphinfish *Coryphaena hippurus*; **g)** snake eel *Ophichthidae* sp.; **h)** sole *Austroglossus* sp.; **i)** longsnout pipe fish *Syngnathus temminckii*; **l)** shyshark *Haploblepharus* sp.; **m)** cuttlefish *Sepia vermiculata*; **n)** common squid *Loligo vulgaris*; **o)** rock lobster *Jasus lalandii*; **p)** two-spotted cricket *Gryllus bimaculatus*.

**Fig. 2** Example of the application (in ImageJ) of the ‘line selection tool’ to measure the linear distances for the three morphometric parameters: (1) eye diameter (E); (2) head width (H) and (3) operculum width (O).

**Fig. 3** Sample-based rarefaction and species accumulation curves for greater crested tern diet at Robben Island. Accumulation curves show the observed species accumulation from 1510 photo-samples (orange points) and 198 regurgitations (blue points) collected on 18 and 19 April 2015. Rarefaction curves (solid lines) and 95% asymptotic confidence intervals (shaded areas) are based on 1,000 random permutations (shown as light grey points) of the observed data. The rarefaction curve for regurgitations is extrapolated (blue dashed line) based on a GAM fit to the photo-sampling, assuming an equal species accumulation rate beyond the range of the observed data. Vertical dotted lines show sample sizes of 190 and 1500 used to compare the methods.

**Fig. 4** Accuracy of estimated standard length ( $\widehat{SL}$ ) (y-axis) compared with actual SL values (x-axis) of anchovy from photographs in ImageJ using allometric regressions based on estimates of eye diameter ( $\widehat{E}$ , open orange circles), operculum width ( $\widehat{O}$ , open blue circles), head diameter ( $\widehat{H}$ , purple open circles) and the mean of all three (mean  $\widehat{SL}$ , black closed circles). The mean culmen length of greater crested terns (61.2 mm) was used as the reference length to scale the pixel-based length estimates in ImageJ. The grey dashed line represents 100% accuracy.

**Fig. 5** Frequency distribution of anchovy standard length from photo-samples and regurgitations (A = Robben Island; B = Seal Island).